Detection of somatic mutations in Wilms tumours using gene panel sequencing

Silva C[1], Carpinteiro D[1], Sampaio DA[1], Vieira L[1]

[1]Unidade de Tecnologia e Inovação, Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa

Introduction: Wilms tumour (WT) is an embryonal kidney neoplasia in which the causative mutations are largely unknown. However, approximately one third of patients display somatic mutations in *WT1*, *CTNNB1*, *TP53* and/or *WTX* genes, prompting the design of molecular tests to determine the mutational profile of each patient. In this work we describe a novel molecular assay based on next-generation sequencing (NGS) technology which we used to identify mutations in 36 Portuguese WT patients.

Methods: Design Studio (Illumina) was used to create a sequencing panel of 83 PCR amplicons covering 12.306 bases of exonic sequences of *WT1*, *CTNNB1*, *TP53* and *WTX* genes. Amplicons were prepared from tumour and matched peripheral blood DNA samples (n=73) using a TruSeq Custom Amplicon kit (Illumina). Libraries were sequenced on a MiSeq instrument using paired-end 250 bp reads. Sequence reads were aligned to hg19 human genome reference sequence using MiSeq Reporter software (Illumina). Variants were annotated using publicly available databases.

Results: Data analysis of the constitutional DNA of WT patients showed the existence of 31 germline variants, including 9 variants not described in the human dbSNP database. Comparison of matched tumour samples revealed the presence of 14 putative mutations in 12 patients. The mutations included *WT1* (n=3), *CTNNB1* (n=4), *WTX* (n=5) and *TP53* (n=2). In one patient, concomitant *WT1* and *CTNNB1* mutations were found. Comparison of results with previous Sanger sequencing data for *WT1* and *CTNNB1* in the same samples confirmed 5 out of 7 mutations detected by NGS in which the mutated allele frequency was above 20%.

Discussion: We conclude that gene panel sequencing is a fast and sensitive molecular assay for identification of recurrent somatic mutations in WT. However, because two thirds of patients lack known mutations, other NGS-based approaches such as exome sequencing may be fruitful to identify novel mutations in WT.